Glucocorticoid excess in patients with pheochromocytoma compared to paraganglioma and other forms of hypertension

Constantinescu, Georgiana ; Langton, Katharina ; Conrad, Catleen ; Amar, Laurence ; Assié, Guillaume ; Gimenez-Roqueplo, Anne-Paule ; Blanchard, Anne ; Larsen, Casper K ; Mulatero, Paolo ; Williams, Tracy Ann ; Prejbisz, Aleksander ; Fassnacht, Martin ; Bornstein, Stefan ; Ceccato, Filippo ; Fliedner, Stephanie ; Dennedy, Michael ; Peitzsch, Mirko ; Sinnott, Richard ; Januszewicz, Andrzej ; Beuschlein, Felix ; Reincke, Martin ; Zennaro, Maria-Christina ; Eisenhofer, Graeme ; Deinum, Jaap

Abstract: Context: Catecholamines and adrenocortical steroids are important regulators of blood pressure. Bidirectional relationships between adrenal steroids and catecholamines have been established but whether this is relevant to patients with pheochromocytoma is unclear. Objective: This study addresses the hypothesis that patients with pheochromocytoma and paraganglioma (PPGL) have altered steroid production compared to primary hypertensives. Design: Multicenter cross-sectional study. Setting: Twelve European referral centers. Patients: Subjects included 182 patients with pheochromocytoma, 36 with paraganglioma and 270 primary hypertensives. Patients with primary aldosteronism (n=461) and Cushing syndrome (n=124) were included for additional comparisons. Intervention: In patients with PPGLs, surgical resection of tumors. Outcome measures: Differences in mass spectrometry-based profiles of 15 adrenal steroids between groups and after surgical resection of PPGLs. Relationships of steroids to plasma and urinary metanephrines and urinary catecholamines. Results: Patients with pheochromocytoma had higher ($P<0.05$) circulating concentrations of cortisol, 11-deoxycortisol, 11-deoxycorticosterone and corticosterone than primary hypertensives. Concentrations of cortisol, 11-deoxycortisol and corticosterone were also higher ($P<0.05$) in patients with pheochromocytoma than with paraganglioma. These steroids correlated positively with plasma and urinary metanephrines and catecholamines in patients with pheochromocytoma, but not paraganglioma. After adrenalectomy, there were significant decreases in cortisol, 11-deoxycortisol, corticosterone, 11-deoxycorticosterone, aldosterone and 18-oxocortisol. Conclusions: This is the first large study in patients with PPGLs that supports in a clinical setting the concept of adrenal cortical-medullary interactions involving an influence of catecholamines on adrenal steroids. These findings could have implications for the cardiovascular complications of PPGLs and the clinical management of patients with the tumors.

DOI: https://doi.org/10.1210/clinem/dgaa423

Ann; Prejbisz, Aleksander; Fassnacht, Martin; Bornstein, Stefan; Ceccato, Filippo; Fliedner, Stephanie; Dennedy, Michael; Peitzsch, Mirko; Sinnott, Richard; Januszewicz, Andrzej; Beuschlein, Felix; Reincke, Martin; Zennaro, Maria-Christina; Eisenhofer, Graeme; Deinum, Jaap (2020). Glucocorticoid excess in patients with pheochromocytoma compared to paraganglioma and other forms of hypertension. Journal of Clinical Endocrinology Metabolism, 105(9):e3374-e3383.
DOI: https://doi.org/10.1210/clinem/dgaa423

Glucocorticoid excess in patients with pheochromocytoma compared to paraganglioma and other forms of hypertension

Georgiana Constantinescu[1,2], Katharina Langton[3], Catleen Conrad[3], Laurence Amar[4,5,6], Guillaume Assié[6,7,8], Anne-Paule Gimenez-Roqueplo[5,6,9], Anne Blanchard[6,10], Casper K Larsen[5], Paolo Mulatero[11], Tracy Ann Williams[11,12], Aleksander Prejbisz[13], Martin Fassnacht[14,15], Stefan Bornstein[1,16,17], Filippo Ceccato[18], Stephanie Fliedner[19], Michael Dennedy[20], Mirko Peitzsch[3], Richard Sinnott[21], Andrzej Januszewicz[13], Felix Beuschlein[12,17], Martin Reincke[12], Maria-Christina Zennaro[5,6,9], Graeme Eisenhofer[1,3], Jaap Deinum[1,22]

[1]Department of Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany
[2] Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania
[3]Institute of Clinical Chemistry and Laboratory Medicine & University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany
[4]Hôpital Européen Georges Pompidou, Hypertension Unit, APHP, Paris, France;
[5]Cardiovascular Research Center INSERM, UMRS_970, Paris, France;
[6] Paris Descartes University, Sorbonne Paris Cité, Paris, France;
[7]Department of Endocrinology, Center for Rare Adrenal Diseases, Hôpital Cochin, APHP, Paris, France.
[8]Institut Cochin, INSERM, U1016, CNRS 8104, Paris, France;
[9]Hôpital Européen Georges Pompidou, Genetics Unit, Paris, France
[10]INSERM, Centre d'Investigations Cliniques 9201, Paris, France
[11]Division of Internal Medicine and Hypertension, Department of Medical Sciences, University of Turin, Italy
[12]Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Germany
[13]Department of Hypertension, Institute of Cardiology, Warsaw, Poland
[14]Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany
[15]Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany
[16]Division of Diabetes & Nutritional Sciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom
[17]Klinik für Endokrinologie, Diabetologie und Klinische Ernährung, University Hospital, Zürich, Switzerland.
[18]Endocrinology Unit, Department of Medicine DIMED, Padova University Hospital, Padua, Italy
[19]First Department of Medicine, University Medical Center Schleswig-Holstein, Lübeck, Germany
[20] The Discipline of Pharmacology and Therapeutics, School of Medicine, National University of Ireland Galway, Costello Road, Galway, Ireland H91V4AY.
[21]School of Computing and Information Systems, University of Melbourne, Melbourne, Australia
[22]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, the Netherlands.

Abbreviations: PPGL, pheochromocytoma and paraganglioma; BP, blood pressure; PHT, primary hypertensives;

Address for Correspondence: Georgiana Constantinescu, Department of Medicine III, Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany
Email: Georgiana.Constantinescu@uniklinikum-dresden.de

Disclosure: The authors have nothing to disclose.

Sources of funding: This study was supported by grants from the Deutsche Forschungsgemeinschaft (DGE, German Research Foundation, Projektnummer: 314061271-TRR/CRC 205 to TAW; MF;SB; MP; FB; MR; GE; JD) and the European Union Horizon 2020 Program (ENS@T-HT - #633983-2 to KL; LA; GA; APGR; AB; CKL; PM; TAW; SB; MD; RS;FB;MR;MCZ; GE; JD).

Abstract

Context: Catecholamines and adrenocortical steroids are important regulators of blood pressure. Bidirectional relationships between adrenal steroids and catecholamines have been established but whether this is relevant to patients with pheochromocytoma is unclear.

Objective: This study addresses the hypothesis that patients with pheochromocytoma and paraganglioma (PPGL) have altered steroid production compared to primary hypertensives.

Design: Multicenter cross-sectional study

Setting: Twelve European referral centers

Patients: Subjects included 182 patients with pheochromocytoma, 36 with paraganglioma and 270 primary hypertensives. Patients with primary aldosteronism (n=461) and Cushing syndrome (n=124) were included for additional comparisons.

Intervention: In patients with PPGLs, surgical resection of tumors.

Outcome measures: Differences in mass spectrometry-based profiles of 15 adrenal steroids between groups and after surgical resection of PPGLs. Relationships of steroids to plasma and urinary metanephrines and urinary catecholamines.

Results: Patients with pheochromocytoma had higher (P<0.05) circulating concentrations of cortisol, 11-deoxycortisol, 11-deoxycorticosterone and corticosterone than primary hypertensives. Concentrations of cortisol, 11-deoxycortisol and corticosterone were also higher (P<0.05) in patients with pheochromocytoma than with paraganglioma. These steroids correlated positively with plasma and urinary metanephrines and catecholamines in patients with pheochromocytoma, but not paraganglioma. After adrenalectomy, there were significant decreases in cortisol, 11-deoxycortisol, corticosterone, 11-deoxycorticosterone, aldosterone and 18-oxocortisol.

Conclusions: This is the first large study in patients with PPGLs that supports in a clinical setting the concept of adrenal cortical-medullary interactions involving an influence of catecholamines on adrenal steroids. These findings could have implications for the cardiovascular complications of PPGLs and the clinical management of patients with the tumors.

Key words pheochromocytoma, paraganglioma, hypertension, steroids, metanephrines

Introduction

Pheochromocytoma is a tumor arising from chromaffin cells of the adrenal medulla that produces the catecholamines epinephrine and norepinephrine in variable amounts (1). When similar tumors arise from extraadrenal chromaffin cells they are termed paragangliomas and produce mainly norepinephrine and sometimes dopamine.

Hypertension in patients with pheochromocytoma and paraganglioma (PPGL) is considered to mainly result from the vasopressor effects of increased circulating concentrations of norepinephrine and epinephrine. Steroids such as aldosterone and cortisol that originate from the adrenal cortex also increase blood pressure but by different mechanisms such as increased sodium reabsorption in the kidney and actions on the systemic vasculature (2).

An impact of the adrenal cortex on adrenal medullary catecholamine synthesis is well established, starting with observations of over 50 years ago that adrenal synthesis of epinephrine depends on glucocorticoid-mediated induction of phenylethanolamine-N-methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine (3). Differences in PPGLs that produce epinephrine versus those that produce predominantly norepinephrine have since been shown to reflect absence or presence of pseudohypoxia-induced blockade of glucocorticoid-mediated induction of PNMT (4). Although impact of adrenal cortex-derived steroids on adrenal medullary catecholamine systems is well established, it has also become apparent that catecholamines reversely impact steroidogenesis, possibly through paracrine actions (5-8). Moreover, besides short-term regulation by catecholamines there is also a long-term effect involving transcriptional regulation of steroid enzyme expression (9). It thus seems that adrenal medullary and cortical systems are intimately connected (10,11).

The bidirectional connections between the adrenal cortex and medulla raise the question whether increased production of catecholamines in patients with PPGLs might influence adrenal steroidogenesis. We therefore hypothesized that high production of catecholamines in patients with PPGLs may result in alterations in adrenal cortical steroid systems. To explore this hypothesis we applied a mass spectrometry-based method to measure 15 adrenal steroids in plasma of a cohort of

patients with PPGLs compared to additional patients with primary hypertension (PHT). For additional comparative purposes we also included in the analysis groups of patients with primary aldosteronism and Cushing syndrome.

Methods

Patient recruitment

This retrospective cross sectional observational study included 1073 participants recruited under a multicenter protocol (Prospective Monoamine-producing Tumor study) and a registry of the European Network for the Study of Adrenal Tumors (ENSAT). Five groups of patients were recruited: pheochromocytoma (n=182), paraganglioma (n=36), primary aldosteronism (n=461), Cushing syndrome (n=124) and hypertensive volunteers (n=270). Patients were enrolled from 12 European centers: 1. University Hospital Carl Gustav Carus Dresden, Germany; 2. University Hospital of Munich, LMU, Germany; 3. Hôpital Européen Georges Pompidou, Unité d'hypertension, Paris, France 4. Hôpital Cochin, Service d'endocrinologie, Paris, France; 5. Cardiovascular Research Center INSERM, Paris; 6. University Hospital of Turin; 7. Institute of Cardiology, Warsaw, Poland 8. University Hospital of Würzburg, Germany; 9.University Medical Centre Schleswig-Holstein, Lübeck, Germany; 10. Radboud University Medical Centre, Nijmegen, the Netherlands; 11. University Hospital of Padova; 12. University Hospital Galway. Diagnosis was based on results of conventional diagnostic testing following current guidelines (12-14). All study protocols under which patients were recruited were approved by the local ethics committee of each participating center and all subjects provided written informed consent before participation in protocols.

Plasma steroid profiling

All blood samples for plasma steroid profiling were collected in the morning (08:00-11:00) into blood tubes containing lithium heparin or EDTA. Separated plasma was stored at -80°C until the steroid profile was analyzed by LC-MS/MS. All measurements of steroids were performed at a single laboratory in Dresden. The panel included 15 steroids: cortisol, 11-deoxycortisol, 21-deoxycortisol, corticosterone, 11-deoxycorticosterone, aldosterone, 18-oxocortisol, 18-hydroxycortisol, cortisone, progesterone, 17-hydroxyprogesterone, pregnenolone, androstenedione, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS). Full details of the method, including the assay performance characteristics have been described elsewhere (15).

Plasma steroid profiling before and after adrenalectomy

Among the patients with PPGLs, additional plasma samples were available from 100 patients at an interval between 12 and 36 months after surgical removal of tumors (88 with pheochromocytoma and 12 with paraganglioma). Thus, steroid profiles for these patients were obtained in paired samples of plasma at screening before surgical intervention and then again after resection of catecholamine-producing tumors.

Catecholamines and Metanephrines

Biochemical measurements of urinary catecholamines and catecholamine metabolites (metanephrine, normetanephrine) in both urine and plasma were performed at a single laboratory in Dresden using LC-MS/MS as described previously (16,17). Details for blood and urine collections and reference intervals have been described in detail elsewhere (18).

Statistical analysis

Statistical analyses were carried out using JMP statistics software package (SAS Institute Inc, Cary, NC). Due to non-normal distribution of data the non-parametric Kruskal-Wallis and the Steel-Dwass all pairs tests were used for comparisons involving multiple groups. Spearman's rank correlation was used to assess significance of relationships. Significance was defined as $P<0.05$. Reference intervals for steroids depend on age and sex (19). For additional parametric statistical analyses all data were

first logarithmically transformed to normalize distributions and corrected for age and sex using a multivariate model. Multivariate analysis with age and sex as covariates was then carried out to establish significance between groups using the Tukey HSD post-hoc test. Final display of data was achieved from the exponents of least square means to derive geometric means with respective plus and minus standard errors as described elsewhere (20). Within group changes from pre- to post adrenalectomy were calculated using the Wilcoxon signed rank test. The steroids of interest are represented as mean of percentage (%) change with 95% confidence interval (CI), calculated after logarithmic transformation of the fold change and the results re-transformed (antilog) as percentages. Graphics were designed using PRISM 8 (Version 8.2.1).

Results

Patient characteristics

There were equal distributions of sexes in all groups except for patients with Cushing syndrome, in whom the proportions of females were higher ($P<0.0001$) at 77% than in the other four groups in whom proportions varied from 41-56% (Table 1). Patients with paraganglioma were younger ($P<0.05$) than those with pheochromocytoma and primary aldosteronism.

Plasma steroids

As shown in table 2, plasma concentrations of cortisol, 11-deoxycortisol, corticosterone and 11-deoxycorticosterone were all higher ($P<0.05$) in patients with pheochromocytoma than in primary hypertensives. In contrast, 18-hydroxycortisol, DHEAS, androstenedione, pregnenolone and progesterone were lower ($P<0.05$) in patients with pheochromocytoma compared to primary hypertensives. There were also differences between patients with pheochromocytoma and paraganglioma, in who plasma concentrations of cortisol ($P=0.0010$), 11-deoxycortisol ($P= 0.032$) and corticosterone ($P= 0.015$) were higher in those with adrenal than extra-adrenal tumors. DHEA and DHEAS were the only steroids elevated in patients with paraganglioma compared to pheochromocytoma ($P<0.05$).

To account for potential confounding influences of age and sex, we used multivariate analysis to clarify and correct for potential impacts of these variables on the aforementioned differences in plasma steroids between the five groups of subjects- supplemental table 1 (21). This analysis showed negative relationships ($P<0.01$) of age with all steroids except 18-hydroxycortisol and 21-deoxycortisol. There were also variable sex differences, which were particularly pronounced for DHEAS, 17- hydroxyprogesterone and progesterone ($P<0.001$).

With multivariate corrections for age and sex, including generation of least square means and post hoc analyses using the Tukey HSD test, most of the differences observed for the data in table 2 were maintained or new differences realized (Figure 1). In particular, patients with pheochromocytoma showed higher ($P<0.05$) plasma concentrations of cortisol, 11-deoxycortisol, corticosterone and 11-deoxycorticosterone than primary hypertensives. In contrast, patients with pheochromocytoma showed lower ($P<0.05$) plasma concentrations of androstenedione and DHEAS than primary hypertensives.

To put the findings on steroids in PPGL patients in perspective, we also describe patients in who altered steroid synthesis is the primary culprit for their disorder (i.e. patients with hypercortisolism and primary aldosteronism). Patients with Cushing syndrome were distinguished from other groups by elevated ($P<0.05$) plasma cortisol, 11-deoxycortisol and corticosterone, whereas patients with primary aldosteronism were characterized by markedly elevated ($P<0.05$) plasma concentrations of aldosterone and 18-oxocortisol compared to all other groups (Figure 1). Patients with primary aldosteronism and Cushing syndrome both showed similarly higher ($P<0.05$) plasma concentrations of 11-deoxycorticosterone and 18-hydroxycortisol compared to primary hypertensives and pheochromocytoma. Patients with pheochromocytoma and primary aldosteronism showed similarly increased ($P<0.05$) plasma concentrations of 11-deoxycortisol and corticosterone above concentrations in primary hypertensives. However, while cortisol was higher ($P<0.05$) in patients with pheochromocytoma than those with primary aldosteronism, 11-deoxycorticosterone was higher ($P<0.05$) in patients with primary aldosteronism than pheochromocytoma. Apart from DHEAS, which

showed higher concentrations in primary hypertensives than other groups, there were no other steroids remarkably higher in this group than others.

Similar to the differences with primary hypertensives, patients with pheochromocytoma showed higher ($P<0.05$) plasma concentrations of cortisol, 11-deoxycortisol and corticosterone than patients with paraganglioma (Figure 2). There were no other significant differences in measured plasma steroids between these two groups of patients (data not shown).

Pre- and post-operative differences in steroids

Following adrenalectomy, patients with pheochromocytoma presented with decreases in several steroids (Figure 3): cortisol, 11-deoxycortisol, 11-deoxycorticosterone, aldosterone, 18-oxocortisol ($P<0.0001$) and corticosterone ($P=0.0002$). In contrast, DHEA, androstendione, DHEAS, 18-hydroxycortisol were not decreased. Pre- to post-surgical changes in steroids were present only in patients with pheochromocytoma. Patients operated for paraganglioma (12 patients) showed no post-operative decreases in steroids, except cortisone ($P= 0.0186$) (results not shown).

Indices of catecholamine excess in patients with pheochromocytoma and paraganglioma

Urinary outputs of norepinephrine were not significantly different between patients with paragangliomas and pheochromocytoma (Table 3). In contrast, and as further explained in the supplement (21), urinary outputs of normetanephrine, metanephrine and epinephrine and plasma concentrations of normetanephrine were lower in patients with paraganglioma than pheochromocytoma.

Relationships of plasma steroids with indices of catecholamine excess

Among patients with pheochromocytoma, plasma concentrations of 11-deoxycorticosterone and corticosterone were positively ($P<0.02$) related with urinary norepinephrine, the summed total of urinary catecholamines (i.e., norepinephrine and epinephrine) as well as plasma and urinary free normetanephrine and summed total metanephrines (i.e. normetanephrine and metanephrine) in both plasma and urine (Table 4). Weaker relationships were observed for cortisol and 11-deoxycortisol; the former correlated positively ($P<0.05$) with summed total of plasma and urinary metanephrines as well

as urinary catecholamines. In contrast, 11-deoxycortisol was only positively ($P<0.01$) related to urinary normetanephrine and summed totals of urinary metanephrines. There were no relationships between metanephrine and epinephrine with any of the steroids. Furthermore, for patients with paragangliomas there were no significant relationships of any steroid with any of the various measures of catecholamine excess (results not shown).

Discussion

This study presents novel data establishing increased circulating glucocorticoids in patients with pheochromocytoma but not paraganglioma. Among the steroids that were increased in patients with pheochromocytoma, 11-deoxycortisol, 11-deoxycorticosterone and corticosterone showed larger relative increases compared to cortisol; this pattern has also been observed in clinical and subclinical Cushing syndrome where the same steroids provide larger diagnostic signals than cortisol (20,22). Moreover, positive relationships were also observed between several glucocorticoids and plasma and urinary markers of catecholamine excess, thus supporting in a clinical setting the concept advanced from preclinical studies by Ehrhart-Bornstein and Bornstein of bidirectional relationships between catecholaminergic and steroidal systems (11,23,24).

Although isolated cases of pheochromocytoma in association with Cushing Syndrome have been described, these have involved patients with ectopic ACTH secreting pheochromocytoma. (25-27). These patients presented with both clinical signs of Cushing syndrome and biochemically proven hypercortisolism. Cases of subclinical Cushing in patients with pheochromocytoma have also been noted (28-30). This could be of importance, especially pre- and postoperatively, as severe hypoglycemia has been described post-operatively in one of those cases (30). Although hypoglycemia is a frequent post-operative complication in patients with pheochromocytoma, commonly thought to reflect the abrupt post-resection fall in circulating catecholamines (31), it is possible that post-operative changes in glucocorticoids could also be a complicating factor. In this context, it was important to investigate whether plasma glucocorticoid levels decrease after successful surgery. As we have shown, there was a significant decrease in glucocorticoids as well as mineralocorticoids after adrenalectomy. Of course, this could also reflect reduced adrenal cortical reserve. On the other hand,

there were no post-operative decreases in plasma concentrations of adrenal androgens, suggesting that there was at least post-operative compensation of the zona reticularis in the remaining adrenal.

The findings that only patients with pheochromocytoma and not paraganglioma showed increased circulating concentrations of glucocorticoids and that positive relationships of glucocorticoids to indices of catecholamine excess were only observed for patients with pheochromocytoma suggests that it is the locally produced and not the circulating catecholamines that are responsible for the effect. Since metanephrines are produced by metabolism of catecholamines within tumor cells, the more consistent and stronger positive relationships of metanephrines than catecholamines with circulating glucocorticoids also supports a more likely impact of locally derived rather than circulating catecholamines as the driver responsible for the increases in circulating glucocorticoids. Nevertheless, this cannot be firmly established by the present study. It remains possible that there is some influence of circulating catecholamines that might increase adrenal steroidogenesis in a non-paracrine fashion. Alternatively, the positive relationships between indices of catecholamine excess and increased plasma concentrations of steroids might be indirectly related by way of other mechanisms.

We further cannot discriminate between a direct effect of catecholamines on steroid synthesis and an indirect effect mediated through adrenocorticotrophin (ACTH) since we have no data about the latter. Nevertheless, since sustained suppression of ACTH results in reductions of circulating DHEAS (32) the low plasma concentrations of DHEAS in patients with pheochromocytoma appear to be inconsistent with an influence in patients with pheochromocytoma mediated by ACTH. Apart from low ACTH and DHEAS in patients with adrenal Cushing syndrome, other forms of glucocorticoid excess such as subclinical Cushing and primary bilateral macronodular hyperplasia are also associated with reduced plasma concentrations of DHEAS (20,33-35). Thus, since DHEAS is responsive to ACTH, the lowered plasma concentrations of DHEAS might reflect lower plasma concentrations of ACTH, which may result from feedback inhibition of steroids on the hypothalamo-pituitary adrenal axis. Although some animal studies have suggested that catecholamines can directly stimulate ACTH secretion (36), this has not been confirmed in humans (37) and there is thus no evidence to implicate circulating catecholamines in the regulation of ACTH secretion.

As with any endogenous compound measured in plasma, circulating concentrations of steroids reflect both their entry into the circulatory compartment and their clearance from that compartment. Thus, it is conceivable that the elevated plasma concentrations of glucocorticoids in patients with pheochromocytoma, rather than reflecting increases in their production might reflect decreased circulatory clearance. Cortisol has a particularly slow plasma clearance, in part due to the high proportion of the steroid that is bound to transcortin; thus, it is possible that differences in binding to transcortin could also decrease the clearance of glucocorticoids and through this action increase plasma concentrations. Nevertheless, as further detailed in the supplement (21) it seems unlikely that the higher plasma concentrations of glucocorticoids, lower concentrations of DHEAS and unchanged concentrations of most other steroids in patients with pheochromocytoma could reflect divergent effects on clearance or protein-binding rather than an effect on production.

Although elevated plasma concentrations of the three glucocorticoids - cortisol, 11-deoxycortisol and corticosterone – in patients with pheochromocytoma were nowhere close to the higher concentrations in patients with Cushing syndrome, they were similar, or even higher in the case of cortisol, to concentrations in primary aldosteronism. Of relevance to these findings are those of Arlt et al (38), who showed using mass spectrometry-based urinary steroid profiling that patients with primary aldosteronism were characterized by increased urinary outputs of not only free cortisol, but also tetrahydro-11-deoxycorticosterone and tetrahydrocortisone. The latter two steroid metabolites are respectively produced from 11-deoxycorticosterone and corticosterone. Thus, the present findings in plasma support the earlier findings in urine. As further detailed in the supplement (21), lack of increase in plasma cortisol in the present study, but increased urinary free cortisol in the earlier study of Arlt et al (38) is easily explained by the inferiority of morning plasma cortisol compared to the other three plasma corticosteroids and urinary free cortisol for assessing hypercortisolism. Since production of excess cortisol in primary aldosteronism is now recognized as a potential contributing factor to the excess morbidity of primary aldosteronism (39,40), it seems possible that the same may apply to patients with pheochromocytoma.

The strengths of this study include the multicenter inclusion of a large number of patients with different forms of adrenal hypertension as well as large numbers of primary hypertensives. One limitation, in addition to the aforementioned lack of data on ACTH, relates to lack of measurements of 24 hr urinary free cortisol. Also, we had a high proportion of females in the group with Cushing syndrome, as is expected in such patients (41). Although we used multivariate analyses to correct for such influences, it remains possible that this may have been insufficient for some comparisons. Nevertheless, such differences along with the sex imbalance in the patients with Cushing syndrome are not relevant to the higher plasma concentrations of glucocorticoids and aldosterone in patients with PPGLs compared to hypertensive groups.

Pheochromocytomas are among the most life-threatening of all endocrine diseases, with elevated morbidity and mortality, especially if undiagnosed. The diagnosis represents a challenge, due partly to lack of specificity of most clinical signs and symptoms. The cardiovascular complications are mainly secondary to excessive secretion of catecholamines from tumors. However, the present data raises the possibility that glucocorticoids might also contribute to the cardiovascular and metabolic complications caused by pheochromocytomas. This supports the concept that altered steroid concentrations in patients with pheochromocytoma may have clinical consequences.

Acknowledgements: We thank the many varied contributions of the clinical support staff at the multiple contributing medical centers as well the technical assistance of Denise Kaden at Dresden.

References

1. Lenders JWM, Eisenhofer G. Update on Modern Management of Pheochromocytoma and Paraganglioma. *Endocrinol Metab (Seoul)*. 2017;32(2):152-161.
2. Isidori AM, Graziadio C, Paragliola RM, Cozzolino A, Ambrogio AG, Colao A, Corsello SM, Pivonello R. The hypertension of Cushing's syndrome: controversies in the pathophysiology and focus on cardiovascular complications. *J Hypertens*. 2015;33(1):44-60.
3. Wurtman RJ, Axelrod J. Adrenaline synthesis: control by the pituitary gland and adrenal glucocorticoids. *Science (New York, NY)*. 1965;150(3702):1464-1465.
4. Qin N, de Cubas AA, Garcia-Martin R, Richter S, Peitzsch M, Menschikowski M, Lenders JW, Timmers HJ, Mannelli M, Opocher G, Economopoulou M, Siegert G, Chavakis T, Pacak K, Robledo M, Eisenhofer G. Opposing effects of HIF1alpha and HIF2alpha on chromaffin cell phenotypic features and tumor cell proliferation: Insights from MYC-associated factor X. *International journal of cancer*. 2014;135(9):2054-2064.
5. Ehrhart-Bornstein M, Bornstein SR, Guse-Behling H, Stromeyer HG, Rasmussen TN, Scherbaum WA, Adler G, Holst JJ. Sympathoadrenal regulation of adrenal androstenedione release. *Neuroendocrinology*. 1994;59(4):406-412.
6. Ehrhart-Bornstein M, Bornstein SR, Gonzalez-Hernandez J, Holst JJ, Waterman MR, Scherbaum WA. Sympathoadrenal regulation of adrenocortical steroidogenesis. *Endocr Res*. 1995;21(1-2):13-24.
7. Ehrhart-Bornstein M, Hilbers U. Neuroendocrine properties of adrenocortical cells. *Horm Metab Res*. 1998;30(6-7):436-439.
8. Lefebvre H, Prevost G, Louiset E. Autocrine/paracrine regulatory mechanisms in adrenocortical neoplasms responsible for primary adrenal hypercorticism. *Eur J Endocrinol*. 2013;169(5):R115-138.
9. Guse-Behling H, Ehrhart-Bornstein M, Bornstein SR, Waterman MR, Scherbaum WA, Adler G. Regulation of adrenal steroidogenesis by adrenaline: expression of cytochrome P450 genes. *J Endocrinol*. 1992;135(2):229-237.

10. Ehrhart-Bornstein M, Bornstein SR, Scherbaum WA. Sympathoadrenal system and immune system in the regulation of adrenocortical function. *Eur J Endocrinol*. 1996;135(1):19-26.

11. Ehrhart-Bornstein M, Bornstein SR. Cross-talk between adrenal medulla and adrenal cortex in stress. *Annals of the New York Academy of Sciences*. 2008;1148:112-117.

12. Lenders JWM, Duh Q-Y, Eisenhofer G, Gimenez-Roqueplo A-P, Murad MH, Grebe SKG, Naruse M, Pacak K, Young WF, Jr. Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*. 2014;99(6):1915-1942.

13. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF, Jr. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *The Journal of clinical endocrinology and metabolism*. 2016;101(5):1889-1916.

14. Nieman LK, Biller BMK, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*. 2008;93(5):1526-1540.

15. Peitzsch M, Dekkers T, Haase M, Sweep FC, Quack I, Antoch G, Siegert G, Lenders JW, Deinum J, Willenberg HS, Eisenhofer G. An LC-MS/MS method for steroid profiling during adrenal venous sampling for investigation of primary aldosteronism. *J Steroid Biochem Mol Biol*. 2015;145:75-84.

16. Peitzsch M, Prejbisz A, Kroiss M, Beuschlein F, Arlt W, Januszewicz A, Siegert G, Eisenhofer G. Analysis of plasma 3-methoxytyramine, normetanephrine and metanephrine by ultraperformance liquid chromatography-tandem mass spectrometry: utility for diagnosis of dopamine-producing metastatic phaeochromocytoma. *Ann Clin Biochem*. 2013;50(Pt 2):147-155.

17. Peitzsch M, Pelzel D, Glockner S, Prejbisz A, Fassnacht M, Beuschlein F, Januszewicz A, Siegert G, Eisenhofer G. Simultaneous liquid chromatography tandem mass spectrometric determination of urinary free metanephrines and catecholamines, with comparisons of free and deconjugated metabolites. *Clin Chim Acta*. 2013;418:50-58.

18. Eisenhofer G, Peitzsch M, Kaden D, Langton K, Mangelis A, Pamporaki C, Masjkur J, Geroula A, Kurlbaum M, Deutschbein T, Beuschlein F, Prejbisz A, Bornstein SR, Lenders JWM. Reference intervals for LC-MS/MS measurements of plasma free, urinary free and urinary acid-hydrolyzed deconjugated normetanephrine, metanephrine and methoxytyramine. *Clin Chim Acta*. 2019;490:46-54.

19. Eisenhofer G, Peitzsch M, Kaden D, Langton K, Pamporaki C, Masjkur J, Tsatsaronis G, Mangelis A, Williams TA, Reincke M, Lenders JWM, Bornstein SR. Reference intervals for plasma concentrations of adrenal steroids measured by LC-MS/MS: Impact of gender, age, oral contraceptives, body mass index and blood pressure status. *Clin Chim Acta*. 2017;470:115-124.

20. Masjkur J, Gruber M, Peitzsch M, Kaden D, Di Dalmazi G, Bidlingmaier M, Zopp S, Langton K, Fazel J, Beuschlein F, Bornstein SR, Reincke M, Eisenhofer G. Plasma Steroid Profiles in Subclinical Compared With Overt Adrenal Cushing Syndrome. *The Journal of clinical endocrinology and metabolism*. 2019;104(10):4331-4340.

21. Constantinescu G, Langton K, Conrad C. Glucocorticoid excess in patients with pheochromocytoma compared with paraganglioma and other forms of hypertension. *Opara Repository*. 2020. http://dx.doi.org/10.25532/OPARA-85

22. Eisenhofer G, Masjkur J, Peitzsch M, Di Dalmazi G, Bidlingmaier M, Grüber M, Fazel J, Osswald A, Beuschlein F, Reincke M. Plasma Steroid Metabolome Profiling for Diagnosis and Subtyping Patients with Cushing Syndrome. *Clin Chem*. 2018;64(3):586-596.

23. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev*. 1998;19(2):101-143.

24. Schinner S, Bornstein SR. Cortical-chromaffin cell interactions in the adrenal gland. *Endocr Pathol*. 2005;16(2):91-98.

25. Oh HC, Koh JM, Kim MS, Park JY, Shong YK, Lee KU, Kim GS, Hong SJ, Koo HL, Kim WB. A case of ACTH-producing pheochromocytoma associated with pregnancy. *Endocr J*. 2003;50(6):739-744.

26. Nijhoff MF, Dekkers OM, Vleming LJ, Smit JW, Romijn JA, Pereira AM. ACTH-producing pheochromocytoma: clinical considerations and concise review of the literature. *Eur J Intern Med*. 2009;20(7):682-685.

27. Langton K, Gruber M, Masjkur J, Steenblock C, Peitzsch M, Meinel J, Lenders J, Bornstein S, Eisenhofer G. Hypertensive crisis in pregnancy due to a metamorphosing pheochromocytoma with postdelivery Cushing's syndrome. *Gynecol Endocrinol*. 2018;34(1):20-24.

28. Finkenstedt G, Gasser RW, Hofle G, Lhotta K, Kolle D, Gschwendtner A, Janetschek G. Pheochromocytoma and sub-clinical Cushing's syndrome during pregnancy: diagnosis, medical pre-treatment and cure by laparoscopic unilateral adrenalectomy. *Journal of endocrinological investigation*. 1999;22(7):551-557.

29. Takizawa N, Muguruma K, Sasano H. Pheochromocytoma and subclinical Cushing's syndrome with focal adrenocortical hyperplasia. 2011;18(7):548-549.

30. Kastelan D, Ravic KG, Cacic M, Stern-Padovanr R, Coric M, Jelcic J, Perkovic Z, Giljevic Z, Aganovic I, Korsic M. Severe postoperative hypoglycemia in a patient with pheochromocytoma and preclinical Cushing's syndrome. *Medical science monitor : international medical journal of experimental and clinical research*. 2007;13(3):Cs34-37.

31. Araki S, Kijima T, Waseda Y, Komai Y, Nakanishi Y, Uehara S, Yasuda Y, Yoshida S, Yokoyama M, Ishioka J, Matsuoka Y, Saito K, Kihara K, Nakano Y, Yoshimoto T, Uchida T, Fujii Y. Incidence and predictive factors of hypoglycemia after pheochromocytoma resection. *Int J Urol*. 2019;26(2):273-277.

32. McKenna TJ, Fearon U, Clarke D, Cunningham SK. A critical review of the origin and control of adrenal androgens. *Baillieres Clin Obstet Gynaecol*. 1997;11(2):229-248.

33. Yener S, Yilmaz H, Demir T, Secil M, Comlekci A. DHEAS for the prediction of subclinical Cushing's syndrome: perplexing or advantageous? *Endocrine*. 2015;48(2):669-676.

34. Hannah-Shmouni F, Berthon A, Faucz FR, Briceno JM, Maria AG, Demidowich A, Peitzsch M, Masjkur J, Bonnet-Serrano F, Vaczlavik A, Bertherat J, Reincke M, Eisenhofer G,

Stratakis CA. Mass spectrometry-based steroid profiling in primary bilateral macronodular adrenocortical hyperplasia. *Endocr Relat Cancer*. 2020.

35. Dennedy MC, Annamalai AK, Prankerd-Smith O, Freeman N, Vengopal K, Graggaber J, Koulouri O, Powlson AS, Shaw A, Halsall DJ, Gurnell M. Low DHEAS: A Sensitive and Specific Test for the Detection of Subclinical Hypercortisolism in Adrenal Incidentalomas. *The Journal of clinical endocrinology and metabolism*. 2017;102(3):786-792.

36. Mezey E, Reisine TD, Palkovits M, Brownstein MJ, Axelrod J. Direct stimulation of beta 2-adrenergic receptors in rat anterior pituitary induces the release of adrenocorticotropin in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 1983;80(21):6728-6731.

37. Eisenhofer G, Goldstein DS, Stull RW, Gold PW, Keiser HR, Kopin IJ. Dissociation between corticotrophin and catecholamine responses to isoprenaline in humans. *Clin Exp Pharmacol Physiol*. 1987;14(4):337-341.

38. Arlt W, Lang K, Sitch AJ, Dietz AS, Rhayem Y, Bancos I, Feuchtinger A, Chortis V, Gilligan LC, Ludwig P, Riester A, Asbach E, Hughes BA, O'Neil DM, Bidlingmaier M, Tomlinson JW, Hassan-Smith ZK, Rees DA, Adolf C, Hahner S, Quinkler M, Dekkers T, Deinum J, Biehl M, Keevil BG, Shackleton CH, Deeks JJ, Walch AK, Beuschlein F, Reincke M. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. *JCI Insight*. 2017;2(8).

39. Adolf C, Köhler A, Franke A, Lang K, Riester A, Löw A, Heinrich DA, Bidlingmaier M, Treitl M, Ladurner R, Beuschlein F, Arlt W, Reincke M. Cortisol Excess in Patients With Primary Aldosteronism Impacts Left Ventricular Hypertrophy. *The Journal of clinical endocrinology and metabolism*. 2018;103(12):4543-4552.

40. Akehi Y, Yanase T, Motonaga R, Umakoshi H, Tsuiki M, Takeda Y, Yoneda T, Kurihara I, Itoh H, Katabami T, Ichijo T, Wada N, Shibayama Y, Yoshimoto T, Ashida K, Ogawa Y, Kawashima J, Sone M, Inagaki N, Takahashi K, Fujita M, Watanabe M, Matsuda Y, Kobayashi H, Shibata H, Kamemura K, Otsuki M, Fujii Y, Yamamoto K, Ogo A, Okamura S, Miyauchi S, Fukuoka T, Izawa S, Hashimoto S, Yamada M, Yoshikawa Y, Kai T, Suzuki T,

Kawamura T, Naruse M. High Prevalence of Diabetes in Patients With Primary Aldosteronism (PA) Associated With Subclinical Hypercortisolism and Prediabetes More Prevalent in Bilateral Than Unilateral PA: A Large, Multicenter Cohort Study in Japan. *Diabetes Care*. 2019;42(5):938-945.

41. Lindholm J, Juul S, Jorgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, Hagen C, Jorgensen J, Kosteljanetz M, Kristensen L, Laurberg P, Schmidt K, Weeke J. Incidence and late prognosis of cushing's syndrome: a population-based study. *The Journal of clinical endocrinology and metabolism*. 2001;86(1):117-123.

Figure legends

Figure 1. Plasma concentrations for 12 steroids of the 15 steroid panel in patients with pheochromocytoma compared to primary hypertensives and patients with Cushing syndrome and primary aldosteronism. Values of steroids are shown as least square means corrected for age and sex and calculated from exponents of logarithmically transformed data (geometric means) with standard errors.

Figure 2. Plasma concentrations for three glucocorticoids in patients with pheochromocytoma compared to those with paraganglioma. Values are shown as least square means corrected for age and sex and calculated from exponents of logarithmically transformed data (geometric means) with standard errors.

Figure 3. Pre- to post-operative differences in plasma concentrations of 11 steroids after adrenalectomy in patients with pheochromocytoma. Differences in the steroids of interest are shown as geometric means of percentage (%) changes with 95% confidence intervals (CI). Means and confidence intervals were calculated after logarithmic transformation of fold changes and the results re-transformed (antilog) as percentages.

Table 1. Age and sex distribution

Group	PHT	PGL	PHEO	PA	CS
N	270	36	182	461	124
Sex (F/M)	120/150	17/19	109/73	188/273	95*/29
Age±SD	49.2±13.3	44.±14.4*¤‡	50.0±15.0	51.1±10.6	48.5±15.5

Ages are shown as means ± standard deviation,*P< 0.005, ¤different than PA, ‡different than Pheo

Table 2 Plasma concentrations of 15 adrenal steroids in the reference group (PHT) compared to patients with paraganglioma (PGL), pheochromocytoma (PHEO), primary aldosteronism (PA) and Cushing Syndrome (CS)

	Reference Group	Endocrine hypertension			
Plasma steroids (nmol/L)	**PHT**	**PGL**	**PHEO**	**PA**	**CS**
Cortisol	257 (205-341)	248 (213-378)	338***§¥ (259-452)	317***§ (227-412)	496***† (390-636)
11-Deoxycortisol	0.47 (0.29-0.71)	0.47 (0.29-0.93)	0.86***§¥ (0.45-1.55)	0.88***§ (0.54-1.61)	1.53***† (0.86-2.85)
21-Deoxycortisol	0.035 (0.017-0.069)	0.029 (0.017-0.049)	0.043 (0.020-0.089)	0.066***§¥£ (0.028-0.188)	0.052*§¥ (0.020-0.121)
Aldosterone	0.121 (0.068-0.190)	0.127 (0.060-0.219)	0.142 (0.063-0.260)	0.354***† (0.201-0.610)	0.093 (0.034-0.239)
Corticosterone	4.70 (3.05-8.41)	4.20*‡ (2.72-7.38)	6.97***§¥ (4.19-11.82)	6.61***§¥ (3.62-12.15)	10.29***§¤*¥‡ (6.28-17.42)
11-Deoxycorticosterone	0.102 (0.052-0.184)	0.154*§ (0.096-0.223)	0.196***§ (0.098-0.281)	0.218*§¥‡ (0.146-0.345)	0.207***§ (0.134-0.383)
18-Oxocortisol	0.022 (0.013-0.037)	0.027 (0.016-0.069)	0.027 (0.016-0.058)	0.053***† (0.026-0.230)	0.030 (0.011-0.047)
18-Hydroxycortisol	1.60 (1.11-2.28)	1.26*§ (0.83-1.66)	1.34*§ (0.89-1.98)	2.12***§¥‡ (1.28-4.01)	2.16***§¥‡ (1.21-3.53)
Cortisone	49.52 (42.93-58.61)	49.09 (42.50-62.85)	52.98 (44.05-62.69)	49.11*‡ (39.12-59.23)	55.70*§¤ (42.67-70.02)
DHEA	8.37 (5.30-13.90)	11.88*§ (6.73-19.59)	8.40*¥ (4.14-13.32)	7.76*¥ (4.19-12.5)	6.25 (2.03-15.98)
DHEA-SO$_{4(micomol)}$	8.31 (4.61-12.00)	7.63 (4.80-9.27)	4.09***§¥ (2.24-7.78)	5.41*§ (3.24-8.48)	5.82 (1.45-13.80)
Androstenedione	2.97*£ (2.19-4.15)	2.32 (1.85-2.98)	2.23***§ (1.51-3.19)	2.69*‡ (1.78-4.03)	3.83*† (1.95-7.73)
Pregnenolone	3.75 (2.41-6.01)	3.89 (1.77-8.27)	3.17***§ (1.09-5.70)	2.12*§¥ (0.66-6.29)	4.69 *¤ (1.27-10.00)
17-Hydroxyprogesterone	1.70 (0.58-2.60)	1.93 (1.16-3.28)	1.41 (0.65-2.47)	2.01***§‡ (1.05-3.05)	1.41*¤ (0.74-2.71)
Progesterone	0.25 (0.18-0.36)	0.23 (0.07-0.48)	0.12***† (0.05-0.23)	0.25***‡ (0.13-0.50)	0.23*‡ (0.08-0.51)

Plasma concentrations are shown as medians in nmol/L , the 25th and 75th quartiles in paranthesis * P< 0.005, *** P<0.0001, §different than PHT, ¥different thn PGL, ‡ different than PHEO, £different than CS, ¤different than PA,†different than all groups

Table 3: Twenty-four hour urinary outputs of catecholamines and metanephrines and plasma concentrations of metanephrines in patients with pheochromocytoma compared to paraganglioma

	Pheochromocytoma	Paraganglioma	*P*-value
	n=183	n=35	
Urinary NE (nmol/day)	472 (231-1255)[¥]	388 (175-977)[§]	NS
Urinary E (nmol/day)	98 (21-394)	18 (9-28)	<0.001
Urinary NMN (nmol/day)	1332 (523-3387)[¥]	538 (225-1543)[§]	0.0119
Urinary MN (nmol/day)	534 (94-1761)	86 (51-104)	<0.0001
Plasma NMN (nmol/L)	4.26 (1.55-10.7)	2.27 (0.88-6.18)	0.0278
Plasma MN (nmol/L)	0.92 (0.17-2.96)	0.17 (0.13-0.22)	<0.0001

Abbreviations: NE, norepinephrine; E, epinephrine; NMN, normetanephrine; MN, metanephrine; NS, not significant. Results are shown as medians and interquartiles (25th and 75th); the significance for differences were assessed using Wilcoxon sum ranked-test and are shown as P-values; [§] N=29, [¥]N=144

Table 4: Relationships of plasma steroid concentrations with plasma and urinary metanephrines and urinary catecholamines in patients with pheochromocytoma

	cortisol		11-deoxycortisol		11-deoxycorticosterone		corticosterone	
	r_s	p	r_s	p	r_s	p	r_s	p
Plasma metanephrines								
NMN	0.1326	0.0744	0.0730	0.3271	0.2171	0.0032	0.1830	0.0134
NMN+MN	0.1874	0.0113	0.0869	0.2436	0.1916	0.0096	0.2170	0.0033
Urinary free metanephrines								
NMN	0.1487	0.0754	0.2166	0.0091	0.3472	<0.0001	0.2869	0.0005
NMN+MN	0.1907	0.0216	0.2253	0.0064	0.3273	<0.0001	0.3160	0.0001
Urinary catecholamines								
NE	0.1592	0.0575	0.1187	0.1579	0.2840	0.0006	0.2089	0.0123
NE+E	0.2101	0.0118	0.1371	0.1026	0.2589	0.0018	0.2426	0.0035

Relationships are shown for NMN and NE as well as the sums of both NMN and MN or NE and E according to Spearman's rank correlation coefficient (r_s) and significance (p). Abbreviations: NMN, normetanephrine; MN, metanephrine; NE, norepinephrine; E, epinephrine.

Figure 1. Results of the steroid panel in patients with pheochromocytoma compared with patients with other forms of adrenal hypertension

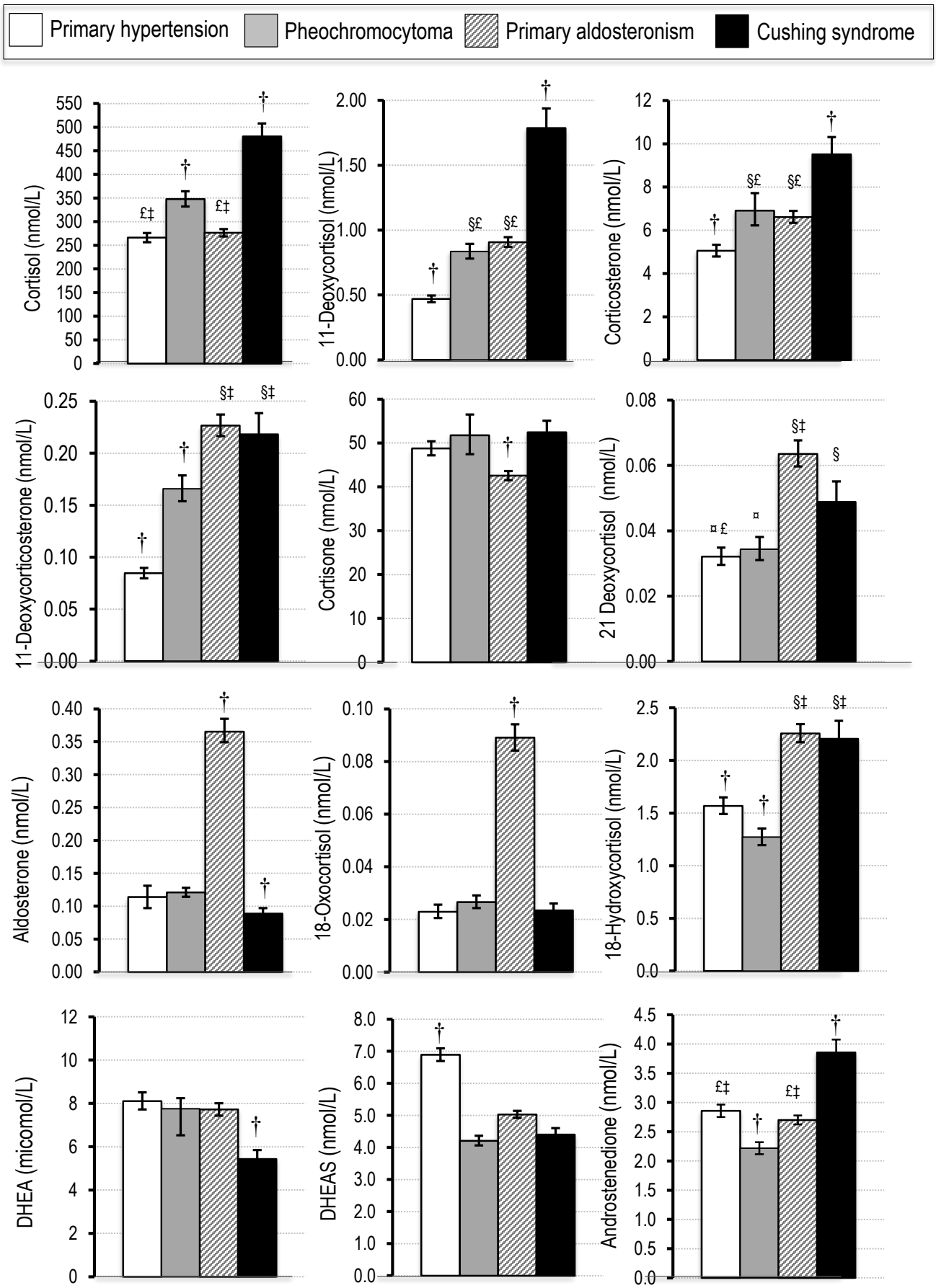


Figure 1. § P< 0.05 different than primary hypertension, ‡ different than pheochromocytoma, £ different than Cushing syndrome, ¤different than primary aldosteronism †different than all groups

Figure 2. Results of the steroid panel in patients with pheochromocytoma compared with patients with paraganglioma

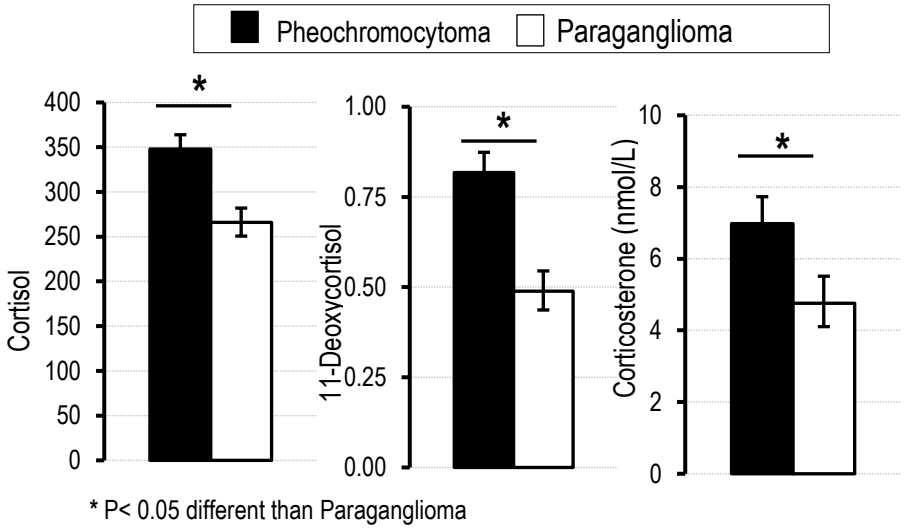
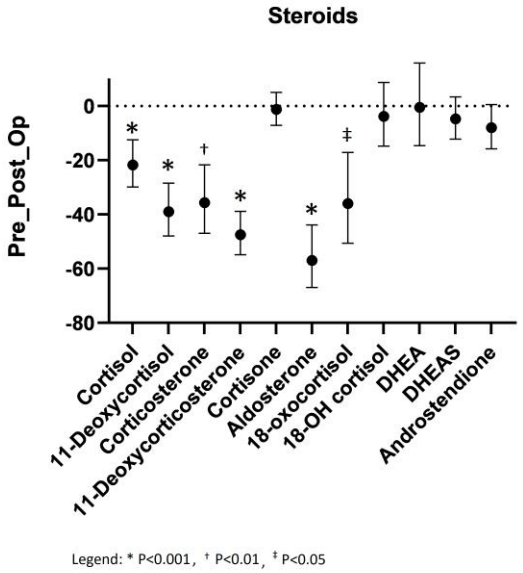


Figure 3. Decrease in steroids after adrenalectomy in pheochromocytoma patients



Legend: * P<0.001, † P<0.01, ‡ P<0.05